High-Density Lipoprotein Function: Recent Advances
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High-Density Lipoprotein Function
Recent Advances
Benjamin J. Ansell, MD, FACC, Karol E. Watson, MD, PtId, Alan M. Fogelman, MD, FACC, Mohamad Navab, PtId, Gregg C. Fonarow, MD, FACC
Los Angeles, California

Although high-density lipoproteins (HDL) possess many features that contribute to the association between elevated HDL cholesterol and protection from atherosclerosis, these lipoproteins may be modified in certain individuals and/or circumstances to become proinflammatory. The ability of HDL to inhibit or paradoxically to enhance vascular inflammation, lipid oxidation, plaque growth, and thrombosis reflects changes in specific enzyme and protein components. The anti-inflammatory and proinflammatory functional properties of HDL can now be assessed using cell-based and cell-free assays. Acute or chronic systemic inflammation and the metabolic syndrome appear to render HDL proinflammatory.

In contrast, statins and experimental agents such as apolipoprotein A-1 mimetics render HDL more anti-inflammatory. Functional characterization of HDL is a promising method for enhanced assessment of cardiovascular risk and effectiveness of risk reduction.

It is well established that the concentration of cholesterol in high-density lipoproteins (HDL) is an inverse predictor of future atherosclerotic cardiovascular disease. A growing body of experimental evidence suggests that augmenting the levels and/or function of HDL and its apolipoproteins can have major vascular protective effects ranging from prevention to stabilization and regression, independent of total or non-high-density lipoprotein cholesterol (HDL-C) levels. This experiment evidence and recent clinical trials have stimulated tremendous interest in the structure, function, and therapeutic potential of HDL.

The best-known antiatherogenic function of HDL is its ability to promote the efflux of cholesterol from cells. However, HDL also has antioxidant, anti-inflammatory, and antithrombotic properties. It has recently been recognized that HDL can both moderate as well as enhance inflammation in atherogenesis. The anti-inflammatory functions of HDL include limiting lipid peroxidation, influencing expression of cytokines, modulating recruitment/adhesion of monocytes, and altering other aspects of endothelial function (1). Paradoxically, HDL particles can also assume proinflammatory, proatherogenic characteristics, especially when an acute phase or chronic systemic inflammatory response is present (2).

Dietary and exercise strategies can lead to modest and variable improvements in HDL-C concentrations and other vascular risk factors, and may be associated with greater antioxidative potential of HDL (3). Both conventional pharmacologic approaches to lipid modification with statins, and novel agents, such as apolipoprotein A-1 (apo-A1) mimetic peptides, can favorably impact the anti-inflammatory/proinflammatory potential of HDL (4); HDL-C levels have limited utility in assessing an individual’s risk of coronary heart disease (CHD). Functional characterization of HDL may allow for enhanced accuracy in assessing cardiovascular risk as well as provide a therapeutic target for risk reduction. In this article, we will review the current understanding of HDL function, its role in atherosclerosis, and the potential clinical utility of assessing HDL function.

HDL structure and role in reverse cholesterol transport. The HDL particles consist of an outer amphipathic layer of free cholesterol, phospholipid, and several apolipoproteins (apo A-I, AII, C, E, AIV, J, and D) on the surface, with a triglyceride and cholesterol ester-rich hydrophobic core. Apolipoprotein A-1 is the principal protein of HDL; HDL particles also carry enzymes, such as paraoxonase, platelet activating factor-acetylhydrolase, lecithin cholesterol acyltransferase, and cholesteryl ester transfer protein (CETP). High-density lipoprotein particles have been characterized into several subtypes on the basis of ultracentrifugation, electrophoresis, or nuclear magnetic resonance; these subtypes include HDL-2 and HDL-3. The differences in particle size results mainly from the number of apolipoprotein molecules and the volume of the cholesterol ester in the core of the particle (5).

The antiatherogenic function of HDL particles that is most commonly appreciated relates to its ability to promote the efflux of cholesterol from cells, a capacity that is generally attributed to apoA-1. A critical function of HDL is to mediate the efflux of cholesterol from vascular macrophages and other peripheral tissues, along with subsequent transfer of cholesteryl ester to plasma or hepatic acceptor proteins. Efflux of cholesterol can occur by several mecha-
High-density lipoprotein (HDL) plays an antioxidant role. HDL protects against the effects of lipid oxidation and provides a means for the removal of cholesterol from peripheral tissues. HDL also has anti-inflammatory and antithrombotic properties that play an important role in the antiatherogenic effects of HDL. The plasma antioxidant capacity and atherothrombosis risk are inversely related. HDL retards monocyte recruitment, expression of oxidized low-density lipoprotein (ox-LDL), and vascular inflammation, and leukocytes from patients with either systemic lupus erythematosus or rheumatoid arthritis show enhanced lipid peroxidation and LDL oxidation (13). These hydroperoxides (products of the 5-lipoxygenase pathway) are bactericidal and cytotoxic (12). The LDL oxidation and plasma lipid peroxide are also increased during systemic infection (14) and the metabolic syndrome (15).

HDL as an antioxidant. High-density lipoprotein plays an important role as an antioxidant by both inhibiting phospholipid oxidation and reducing the activity of minimally modified LDL (16). Several components of HDL contribute to this antioxidant effect, including its major apolipoprotein A-1 along with at least four enzymes, including paraoxanase 1 and lecithin:cholesterol acyltransferase (17). Watson et al. (16) demonstrated that paraoxanase 1 prevents the formation of lipid hydroperoxides and oxidized phospholipids and hydrolyzes them once they are formed. In vitro, apolipoprotein A-1 also reduces lipid hydroperoxides within LDL independent of paraoxanase (18). Graham et al. (19) have shown that phospholipids in the HDL-3 fraction are especially capable of retarding LDL oxidation.

Evidence that HDL can be a pro-oxidant. In many patients with atherosclerosis, it appears that HDL is not only ineffective as an antioxidant, but it paradoxically increases lipid peroxide formation. In addition to being unable to retard LDL oxidation, HDL from patients with a history of CHD enhances the oxidation of LDL and phospholipids in LDL (20). Oxidation of HDL per se does not necessarily generate ineffective HDL, however. MacDonald et al. (21) reported that tyrosyl radical oxidation of mouse HDL enhances its ability to promote cholesterol efflux in vitro and inhibits aortic lesion development better than control HDL or saline (p < 0.001).

While tyrosyl radical oxidation of HDL can augment cholesterol efflux, oxidation and subsequent nitration of HDL have the opposite effect. An antioxidant under normal conditions, endothelial nitric oxide can be altered by myeloperoxidase from phagocytes to create nitric-oxide-derived oxygen species that can promote oxidative nitration and halogenation products within a developing atheroma (22-23). Zheng et al. (22) identified apolipoprotein A-1 as a specific target of myeloperoxidase that, when oxidized and nitrated, impaired ABCA1-dependent cholesterol efflux.

Nitrotyrosine and/or chlorotyrosine modification of apolipoprotein A-1 is inversely correlated with HDL's ability to promote efflux (22). Bergt et al. (24) showed that HDL from the blood of human subjects with coronary disease contains elevated levels of 3-chlorotyrosine compared to circulating HDL from healthy subjects. Furthermore, exposing HDL or apolipoprotein A-1 to the myeloperoxidase product hydrochlorous acid almost entirely prevented ABCA1-dependent reverse cholesterol transport. Figure 1 summarizes the role of myeloperoxidase in catalyzing oxidative modification of HDL, rendering it unable to effect ABCA1-mediated transport.

Analogous to its adverse effects on HDL function, myeloperoxidase may also inhibit endothelial cell function, given the strong inverse relationship between flow-mediated dilation of the brachial artery and serum myeloperoxidase level (25). Based on its potential linkage between oxidation and vascular inflammation, Nicholls and Hazen (23) have suggested that myeloperoxidase itself may be an important biomarker of atherosclerotic risk as well as a target for therapeutic intervention.

HDL retarding monocyte recruitment, expression of monocyte chemotactic protein (MCP)-1 and other cytokines. In response to the production of oxidized phospholipids within the subendothelial space of a developing atherosclerotic plaque, MCP-1 is produced by the vascular

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**Abbreviations and Acronyms**

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<tr>
<th>Abbreviation</th>
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<th>Definition</th>
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<tr>
<td>ABCA1</td>
<td></td>
<td>adenosine triphosphate-binding cassette transporter A1</td>
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<td>apoA-1</td>
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<td>apolipoprotein A-1</td>
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<td>CETP</td>
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<td>cholesteryl ester transfer protein</td>
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<td>CFA</td>
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<td>cell-free assay</td>
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<td>CHD</td>
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<td>HDL</td>
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<td>HDL-C</td>
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<td>LDL</td>
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<td>MCA</td>
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<td>MCP</td>
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<td>ox-LDL</td>
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nisms, including: 1) passive diffusion of free cholesterol from the macrophage, with subsequent esterification by lecithin:cholesterol acyltransferase associated with HDL; 2) transport of cholesterol to HDL via scavenger receptor B1 on the vessel wall surface; and 3) most significantly, binding of lipid-poor apolipoprotein A-1 to the ATP-binding cassette transporter A1 (ABCA1) transporter in the vessel wall, where it accepts free cholesterol, forming pre-beta HDL that matures through esterification to alpha migrating HDL (6). In addition to its role in reverse-cholesterol transport, HDL also has antioxidant, anti-inflammatory, and antithrombotic properties that play an important role in the antiatherogenic effects of HDL.
endothelial cells (26). There is therefore linkage between the production of oxidized lipids and cellular inflammation, and anti-inflammatory HDL appears to moderate both of these processes.

Another important anti-inflammatory role of HDL is to limit the expression of cytokines such as tumor necrosis factor-alpha, and interleukin-1 that mediate up-regulation of leukocyte endothelial adhesion molecules. Cockerill and Barter (27) have shown that human endothelial cells, when pretreated with HDL, show markedly reduced expression of leukocyte adhesion molecules when stimulated by these inflammatory cytokines.

Paradoxical enhancement of MCP-1 expression by HDL. Conversely, HDL can also act to amplify vascular inflammation. Ansell et al. (20) demonstrated that the HDL from 77% of statin-naive patients with CHD or CHD risk equivalents enhanced LDL-induced recruitment of monocytes in an experimental co-culture of endothelial and smooth muscle cells. This contrasted with none of the age-/gender-matched controls from whom proinflammatory HDL was identified using this monocyte chemotaxis assay (MCA) (20). The “inflammatory index” comparing the effects of LDL with and without HDL (defined as 1.0) was 1.38 ± 0.91 in patients versus 0.38 ± 0.14 in controls (p = 0.000015). Thus, the inflammatory/anti-inflammatory properties of HDL distinguished CHD patients from controls better than did HDL-C concentration, which was more comparable between the control and CHD groups (20).

Similar results were seen using a cell-free assay (CFA) comparing the ability of HDL from CHD patients and controls to retard phospholipid oxidation (20). In this analysis, the HDL from 96% of CHD patients was proinflammatory (paradoxically enhanced phospholipid oxidation compared with the production of oxidized phospholipid in the absence of HDL), while none of the controls exhibited proinflammatory HDL by the CFA (20). Using the CFA, the inflammatory index of the patients was 1.19 ± 0.19 compared to that for the controls at 0.53 ± 0.15 (20).

Relationship of anti-inflammatory/proinflammatory properties of HDL to reverse cholesterol transport. The ability of HDL to facilitate cellular cholesterol efflux may be directly related to its anti-inflammatory/inflammatory properties. In the Ansell et al. (28) study, the ability of a subject’s HDL to inhibit monocyte chemotaxis was highly correlated with its capacity to promote cholesterol efflux. Impaired reverse cholesterol transport by HDL leads to increased levels of atherogenic lipoproteins and increased levels of oxidized lipids, which are themselves proinflammatory (29).

Modifiers of HDL anti-inflammatory/inflammatory activity. The potential for HDL to inhibit lipid oxidation and/or monocyte recruitment appears to be modifiable by the acute phase response, medications, and HDL mimetics.

Acute phase response HDL. The acute phase response has both quantitative and qualitative effects on HDL and its constituents (30). Rohrer et al. (31) reported that inflammation reduces HDL-C by increasing the activities of endothelial lipase and soluble phospholipase A2 and by replacing apoA-1 in HDL with serum amyloid A. As examples, sepsis or influenza infection can decrease the concentration of HDL-C in humans by as much as 50% (32).

Inflammation also causes significant changes in the protein and lipid composition of HDL (Fig. 2). In a mouse model, Van Lenten et al. (33) demonstrated that acute influenza infection impaired HDL’s anti-inflammatory capabilities by reducing levels of paraoxonase and platelet-activating factor acetylhydrolase, while increasing cerulo-
plasmin, and apolipoprotein J within HDL. In humans undergoing elective laparoscopic cholecystectomy, levels of paraoxonase and lecithin:cholesterol acyltransferase fell 24% and 44%, respectively, to a nadir three to six days postoperatively (34). These alterations in antioxidative capacity allow for increased production of ox-LDL and minimally modified LDL that contributes to the production of inflammatory cytokines by smooth muscle and endothelial cells.

Pharmacologic modulation: statin effect. In contrast to the effects of systemic inflammation, statin therapy appears to lessen the proinflammatory potential of HDL. While statin treatment is associated with a relatively minimal effect on HDL-C concentration, changes in HDL’s anti-inflammatory/inflammatory potential may be greater. In the Ansell et al. (20) study in the preceding text, after six weeks of treatment with simvastatin 40 mg daily, HDL became anti-inflammatory in 46% (vs. 23% at baseline) of CHD risk equivalent patients’ cases by the MCA and 58% (vs. 4% at baseline) by the CFA. This suggests that conventional statin treatment in CHD patients is associated with enhancement of HDL’s ability to limit monocyte chemotaxis and phospholipid oxidation. However, the inflammatory index of HDL from these statin-treated patients (1.08 ± 0.71) still did not reach the very anti-inflammatory level (0.38 ± 0.14) seen in the controls, as shown in Figure 3 (20).

Pharmacologic modulation: HDL and its analogues. Success of traditional pharmacotherapy directed toward HDL has largely been measured by increases in HDL-C concentration, which can be difficult to achieve in practice. It is assumed that an increase in HDL-C will lead to reduction in CHD events, such as the robust treatment effects that were seen in the HDL Atherosclerosis Treatment study and the Veterans Administration HDL Intervention trial (35,36). Although post-hoc analysis of the latter trial’s results suggested that the 6% increase in HDL-C was the only predictor of response to gemfibrozil, this analysis: 1) showed that only 23% of the reduction in CHD could be attributed to the rise in HDL-C; and 2) did not consider other potentially beneficial effects, including qualitative changes in LDL and/or HDL (37).

Emerging strategies to raise HDL-C to a greater extent than currently possible, such as the 40% to 61% shown with the CETP inhibitor torcetrapib in combination with atorvastatin, are promising but still require demonstration of clinical benefits (38). The relationship between CETP and atherosclerosis is complex, and both proatherogenic and antiatherogenic effects have been suggested; CETP may be atherogenic because of its ability to transfer cholesterol ester from HDL to LDL/very LDL, thus lowering plasma levels of HDL while increasing the levels of LDL/VLDL. Conversely, CETP may also be antiatherogenic by facilitating the production of lipid-poor pre-HDL particles, which are efficient stimulators of reverse cholesterol transport. Both experimental and human epidemiological studies have provided conflicting data (39).

Infusions of HDL and its analogues have shown anti-inflammatory effects and regression of atherosclerosis. Angelin et al. (40) reported that intravenous treatment of humans with pro-apoA-I liposomal complexes resulted in
increased fecal sterol content without an increase in cholesterol synthesis, suggesting a significant increase in reverse cholesterol transport. Reports of similar infusions in humans have shown a significant increase in pre-beta HDL levels, evidence of enhanced reverse cholesterol transport (41), and a significant reduction in the inflammatory properties of LDL (42). Badimon et al. (43) published the first evidence that infusion of HDL allowed for regression of existing atherosclerosis in a rabbit model.

As apoA-1 contains 243 amino acids and requires intravenous infusion, it has limited clinical utility. Human carriers of a naturally occurring variant, apoA-1 Milano, have surprising longevity and apparently little atherosclerosis despite low HDL-C levels (44). Recombinant apoA-1 Milano/phospholipid complexes administered in five weekly intravenous infusions to a cohort of 47 human patients with acute coronary syndromes resulted in a 4.2% reduction in atheroma volume from baseline, as determined by intravascular ultrasound (p < 0.001); placebo treatment had no significant effect (45). The long-term effects and practicality of such a strategy remain to be demonstrated, but the rapid regression in human atherosclerotic plaque seen in this trial is unprecedented.

The search for an orally active apo-A1 mimic that improves HDL function yielded D-4F, an 18 D-amino acid amphipathic peptide able to withstand gastrointestinal enzymes that digest L-amino acids. The order and orientation of these amino acids within this compound are exquisitely critical to its effectiveness in retarding atherosclerosis (46). Navab et al. (47) reported that twice-daily oral ingestion of D-4F in LDL receptor-null mice diminished lesion size by 79% without significant change in plasma cholesterol concentrations. Oral treatment of apo-E–null mice with the peptide also resulted in a marked increase in pre-beta HDL and increased cholesterol efflux from macrophages (48). Plasma from the mice 20 min after receiving oral D-4F demonstrated cholesterol-containing particles similar to micles with high apoA-1 content, paraoxanase activity, and decreased lipid hydroperoxides (48).

Van Lenten et al. (49) also recently demonstrated that D-4F was able to retard the secretion of oxidized phospholipids by pneumocytes in response to influenza infection in vitro, demonstrating that apoA-1 mimetics can have anti-inflammatory properties outside the vasculature. This is consistent with the suggestion by Navab et al. (50) that HDL is part of a system of nonspecific innate immunity. In rodents, infusions of HDL have limited the clinical, biochemical, and histological signs of inflammation from mesenteric ischemia/reperfusion (51,52) and endotoxic shock (52).

**Potential clinical applications of HDL functional testing.** Measurement of HDL anti-inflammatory/inflammatory activity has potential for both defining patients at increased risk of CHD as well as monitoring the effectiveness of therapies capable of modifying these properties. Observing qualitative changes in HDL could assess an individual’s biological response to new therapies such as apoA-1 mimetics, which have minimal/no effect on plasma lipoprotein concentrations. In addition, it will be important to characterize the function of HDL in clinical trials of torcetrapib, while awaiting studies of its effects on atherosclerosis. The functionality of HDL after torcetrapib treatment has not yet been reported but could have important clinical implications, as the antiatherogenic potential of this therapeutic strategy is controversial (53).

Characterizing HDL anti-inflammatory/inflammatory function could also allow for identification of the presumed minority among those patients with elevated HDL-C levels who are at heightened risk for CHD based on an inflammatory HDL phenotype. Most patients with elevated HDL-C concentrations are not candidates for lipid-modifying therapy according to current treatment guidelines, but some nonetheless remain at risk for coronary events (54). Determining HDL function may also identify patients with normal or low HDL levels that are at particularly high risk for cardiovascular events. In addition to identifying a population with dysfunctional HDL that
might possibly benefit from treatments such as apoA-1 mimetics, recognition of proinflammatory HDL might also justify traditional LDL-lowering approaches such as statin therapy in this group, given evidence that statin therapy also moderates HDL dysfunction (2). In individuals with the metabolic syndrome, in whom a constellation of subtle metabolic markers confers increased CHD risk, evaluation of HDL anti-inflammatory/inflammatory activity might provide an integrated assessment of the severity of their increased risk. Such testing might also allow assessment of whether treatment has successfully improved HDL function in an individual patient. It will also be important to incorporate testing of HDL function in clinical trials and studies assessing the risk of future cardiovascular events.

Conclusions. High-density lipoprotein has a variety of functional effects including both proinflammatory and anti-inflammatory properties. The serum HDL-C level does not assess HDL’s functional properties. Even within a normal individual, HDL may become transiently pro-oxidant in the presence of systemic infection. Determination of HDL anti-inflammatory/proinflammatory function will likely yield important additional information beyond that available from simply knowing the quantitative level of HDL-C in an individual. This additional information will likely improve predictive accuracy for CHD and may also provide new strategies for the prevention and treatment of atherosclerosis.

Reprint requests and correspondence: Dr. Gregg C. Fonarow, 47-123 CHS, 10833 LeConte Avenue, Los Angeles, California 90095. E-mail: gfonarow@mednet.ucla.edu.

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